The University of Chicago
Founded by JOHN D. ROCKEFELLER

On the Action of Fehling's Solution on Malt Sugar

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF SCIENCE IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY.

By W. LEE LEWIS

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The following pages contain a report of a preliminary and incomplete investigation carried out by myself, at the suggestion and under the direction of Dr. Nef, of the products formed in the oxidation of malt sugar by alkaline solutions containing cupric hydroxide (Fehling's solution). The work shows that the ratio of the various acids—carbonic, formic, glycollic, hexonic, and probably glycerinic and trioxybutyric—formed in this oxidation differs very decidedly from that observed by Nef[1] in the case of dextrose, laevulose, and mannose, respectively. Furthermore, a new hexonic acid, probably α-oxymethyl-*d*-ribonic, is formed in the case of maltose in place of α-oxymethyl-*d*-arabonic acid.[2] Whereas the simple hexoses named give *d*-gluconic and small amounts of *d*-mannonic acid, malt sugar gives large quantities of glucosido-*d*-mannonic acid only, for reasons already emphasized by Nef.[3] It is certain also that oxidation of maltose by Fehling's solution is not accompanied by any very appreciable hydrolysis into two molecules of *d*-glucose, the main oxidation products formed in this case being glucosido monobasic acids whose separation as such cannot as yet be accomplished. A slight splitting of maltose into two molecules of dextrose before oxidation may possibly occur, since the subsequent hydrolysis of the glucosido acids formed always gave less dextrose than would result if only one-half of the malt sugar molecule, *i. e.*, the aldehyde portion, $C_6H_{12}O_6$, $CH_2OH—CHOH—CHOH—CHOH—CHOH—CHO$, had been oxidized. Thus the hydrolysis of the glucosido acids obtained from 24 grams of maltose which are not affected by boiling Fehling's solution gave in four independent experiments, on heating for 10 hours at 100° with five per cent. sulphuric

[1] Nef: Ann. Chem. (Liebig), **357**, 259.

[2] Nef: Ann. Chem. (Liebig), **357**, 298; Thesis of H. A. Spoehr soon to be published in the American Chemical Journal.

[3] Nef: Ann. Chem. (Liebig), **357**, 232.

acid, a mean of 10.145 grams of *d*-glucose, and three similar experiments with 144 grams of maltose gave 47.67 grams, 55.04 grams, and 48.40 grams dextrose, respectively. Assuming that the malt sugar used contained the usual quantity, one molecule, of crystal water, the amount of *d*-glucose theoretically possible on subsequent hydrolysis of the glucosido acids would be exactly one-half of the sugar originally used. When we bear in mind, however, that *d*-glucose itself is not entirely unchanged on heating with 5 per cent. sulphuric acid at 100°, it is clear from the above statement of facts that the malt sugar molecule oxidizes mainly as such with Fehling's solution, giving glucosido acids. These latter are of especial interest because of their possible bearing on the constitution of malt sugar itself. Furthermore, the well-known fact that one molecule of dextrose, $C_6H_{12}O_6$, takes up 2.48 atoms of oxygen, whereas one molecule of maltose, $C_{12}H_{22}O_{11}$, requires only 2.86 atoms when treated with Fehling's solution, now demands no further explanation.

Perhaps the most striking contrast to be noticed between malt sugar, on the one hand, and the simple hexoses—dextrose, laevulose, and mannose—on the other, is the marked difference in the relative amounts of formic and carbonic acids formed in the treatment with the reagent named. One hundred grams of malt sugar gave 3.60 grams of formic acid and 7.37 grams of carbon dioxide, whereas 100 grams of a simple hexose gave from 2.0 to 4.0 grams of carbon dioxide and from 12.0 to 14.0 grams of formic acid.[1]

The following further difference is also noteworthy: Mannose, dextrose and laevulose gave, besides glycollic and hexonic acids, large amounts of glycerinic and trioxybutyric acids. In the case of maltose, I was unable to establish (by hydrolysis) with certainty the presence of any monobasic acids containing three or four carbon atoms.

It is therefore already certain that the aldehyde portion of the maltose molecule must be oxidized, under the conditions named, in a manner considerably different from that of the oxidation of simple aldohexoses. It would, however,

[1] Nef: Ann. Chem. (Liebig), **357**, 259.

be premature to develop any theory on this point until certainty has been gained as to the complete nature and relative amounts of the various oxidation products formed from malt sugar.[1]

EXPERIMENTAL PART.

Six 24-gram lots of Kahlbaum's maltose, melting at 106°–112°, were worked up in the following manner: Twenty-four grams of maltose were dissolved in 70 cc. of water, and solutions containing 95.6 (2.86 mols. O) grams of hydrated copper sulphate in 300 cc. and 181.0 grams of crystallized barium hydroxide in 750 cc. of water were also prepared. The quantity of copper sulphate used was that just reduced by the 24 grams of maltose under the conditions, and is practically identical with the ratio in use in titrating maltose with Fehling's solution in the usual manner. The barium hydroxide taken was one and a half molecules, calculated against the copper sulphate. Preliminary attempts with three molecules of sodium hydroxide in place of the barium hydroxide failed because of the difficulty of recovering the acids quantitatively from the resulting salt mixture.

All three solutions were heated to boiling and the unfiltered barium hydroxide solution was added slowly with active shaking to the solution of copper sulphate. The hot solution of sugar was similarly added to the mixture and the whole heated in a boiling water bath for one hour with occasional shaking. Reduction, which always started immediately and proceeded rapidly, was complete in this time, as evidenced in the almost entire disappearance of the copper-blue color from the solution. The calculated amount of sulphuric acid was now added, the mixture filtered, and the well-washed, bulky precipitate twice taken up and boiled in 500 cc. of water containing two grams of sulphuric acid. The collected filtrates, which showed no reduction of Fehling's solution, were freed from copper by saturation with hydrogen sulphide, then a very slight excess of barium added, and the water

[1] Work on the further determination of the nature of the products formed from maltose by Fehling's solution, as well as by the oxidation of alkaline solutions of malt and milk sugar by air and by hydrogen peroxide, is at present being continued by Dr. Nef and his students in this laboratory.

and volatile formic acid distilled off at 12 to 25 mm. pressure, the solution being finally heated for one-half hour on the boiling water bath. The residue of acids appeared as a golden brown, slightly mobile syrup. Six such operations with a total of 144 grams of maltose, corresponding to 136.8 grams of the anhydrous sugar, gave 132.8 grams of nonvolatile acids.

Determination of Carbon Dioxide.—Carbon dioxide was determined in independent experiments as follows: Solutions of maltose and copper sulphate were prepared as at first and also a solution of sodium hydroxide containing 46.1 grams (three molecules) in 400 cc. of water. The solutions were well mixed cold—the alkali with the copper sulphate and the sugar with this mixture—and finally boiled in a water bath for one and one-half hours. After filtering off the cuprous oxide the carbon dioxide was set free with hydrochloric acid, calculated for one-third of the alkali used, and driven out of the solution by heating in a flask connected successively with a reversed condenser and with three tall cylinders filled with a saturated aqueous solution of barium hydroxide. The acid was added slowly by means of a separatory funnel, and to insure complete expulsion of the soluble gases, the liquid was finally heated in a bath at 110°–120° for two hours. During the experiment, air freed from carbon dioxide was drawn slowly through the solution. The precipitated barium carbonate was then filtered off, well washed, and dried at 100°. Control experiments were run for the amount of carbon dioxide in the reagents used, and proper deductions made. Twenty-four grams of sugar gave 7.93 grams of barium carbonate, corresponding to 1.77 grams of carbon dioxide.

Determination of Volatile Acids.—The distillates from each 24-gram lot of maltose, amounting to 3.5-4 liters, were separately made up to definite volume and an aliquot portion titrated with N/10 sodium hydroxide and phenolphthalein as an indicator. These results were checked by the method of H. C. Jones[1] for determining formic acid quantitatively which depends upon its conversion in alkaline solution into

[1] Am. Chem. Jour., **17**, 537. Cf. Lieben: Monats. Chem., **14**, 146; and Péan de St. Gilles: Ann. Chim. Phys., [3] **55**, 388.

carbonate with N/10 potassium permanganate. The six distillates averaged 0.790 gram of formic acid with N/10 alkali, and 0.877 gram by Jones's method. These results establish that formic acid is the only volatile acid formed in the experiment, the higher value with permanganate resulting from traces of hydrogen sulphide or other reducing matter carried over in the distillation. Taking the first figures, 144 (138.8 grams of anhydrous sugar) grams of maltose gave 4.740 grams of formic acid.

Determination of Nonvolatile Acids, including Sugar, after Hydrolysis.—The above named residue of nonvolatile syrupy glucosido acids, weighing 132.8 grams, was hydrolyzed by heating under a reflux condenser on the boiling water bath for ten hours with 5 parts of 5 per cent. sulphuric acid. The theoretical amount of barium hydroxide for the removal of the acid was now added, the solution further boiled a half hour, and filtered. After preliminary titrations with Fehling's solution the filtrate was made up to definite volume of approximately one per cent. dextrose content, and accurately titrated for the sugar split off; 50 cc. of Fehling's solution required 24.75 cc. of the 5 liters of filtrate, corresponding to 0.2356 gram of dextrose, or a total of 48.40 grams for the original 132.8 grams of acids.

For the removal of the dextrose the solution was concentrated to 1 liter and boiled in a water bath for 10 hours with 50 grams of powdered calcium carbonate. Twenty-seven grams of undissolved carbonate of lime were filtered off; digestion of this with 5 per cent. acetic acid until there was no further action left an insoluble residue which was dissolved in hydrochloric acid and reprecipitated with ammonia. This gave 0.50 gram of white calcium oxalate; a previous experiment with the same quantities gave 0.9 gram.

Analysis of the salt, dried to constant weight at 100°, gave the following result: 0.4995 gram gave, on ignition, 0.1915 gram CaO.

	Calculated for $CaC_2O_4.H_2O$.	Found.
CaO	38.39	38.35

The aqueous solution of calcium salts and dextrose was concentrated in half-liter hemispherical evaporating dishes and alcohol was added slowly, with thorough stirring. The alcohol was decanted and the process repeated, the black, tarry lime salts being occasionally taken up in water and decolorized with animal charcoal, etc., until finally by precipitation and continued rubbing the salts became granular.[1] After drying they were repeatedly extracted with absolute alcohol until 0.5 gram showed no reduction on boiling with 10 cc. of Fehling's solution. The plate-dried lime salts weighed 107.75 grams and on analysis, by ignition, showed 15.21 per cent. of calcium oxide. The calcium was removed in the usual manner by treating the salts in a hot, dilute solution with a faint excess of oxalic acid. On distilling off the water from the filtrate under reduced pressure, finally heating for one-half hour on the boiling bath, a residue of 82.0 grams of syrupy, slightly mobile acids was obtained, which dissolved entirely in 1 liter of hot, absolute alcohol on long digestion. On concentrating this solution to about 500 cc. there were deposited in 24 hours 12.5 grams of mannonic lactone, melting at 148°–149°. By concentrating the mother liquor, etc., two further crops were obtained, so that finally a total of 22.2 grams of *d*-mannonic lactone were obtained, leaving a residue of 59.8 grams of nonvolatile gummy acids.

A portion of the mannonic lactone dissolved on digestion in 20 parts of absolute alcohol, and on cooling 80 per cent. of the material separated out in the form of light, flaky, snow-white needles. After four crystallizations from absolute alcohol the substance melted at 149°–152° and gave $[\alpha]_{20}^{D} = +51.5$.[2] $d = 1.034$; $p = 10.000$, *i. e.*, 1.7186 grams lactone and 15.4674 grams water. $(\alpha) = +5°.34$ in a 1 dcm. tube.

The substance gave no change of rotation on standing in water solution 24 hours, and all attempts to raise the specific rotation to that given by Fischer, +54°.8, failed.[3]

Mannonic lactone dissolved in twice its weight of 50 per cent.

[1] E. Fischer: Ber. d. chem. Ges., **22,** 363.
[2] Fischer: Ber. d. chem. Ges., **22,** 3222. Nef: Ann. Chem. (Liebig), **357,** 270.
[3] Cf. Nef: Ann. Chem. (Liebig), **357,** 271.

alcohol yields with an equal amount of phenylhydrazine, on standing 24 hours at room temperature, leafy crystals of the acid hydrazide, melting at 216°. It was also further identified by its conversion into the characteristic brucine salt which separates out from concentrated water solution on addition of alcohol in bulky, silky needles melting at 210°–212°. The remaining 59.8 grams of nonvolatile, gummy acids were now resolved into three fractions, A, B and C, as follows: They were first dissolved in an equal weight of absolute alcohol, and twice the volume of ether was thereupon slowly added with much shaking. On standing overnight, decanting, and washing the residue with alcohol-ether (1 to 2 by volume) 26.1 grams of thick gum remained undissolved. The soluble portion, 33.8 grams, as well as the insoluble portion, gave on prolonged digestion with acetic ether a total of 25.8 grams of insoluble gum (C). The gums which dissolved in acetic ether were further resolved (after removing the solvent, etc.), by digestion, into an ether-soluble fraction, A, 11.1 grams, and a portion, B, 25.0 grams.

Fraction C (least soluble gums).—This fraction was titrated by the following method, which was uniformly used throughout the work where acids forming lactones were believed to be present. The material was dissolved in water and made up to a convenient volume and an aliquot portion containing about 0.5 gram gum was then taken for titration. This was considerably diluted with water and treated with an excess of N/10 sodium hydroxide for ten minutes on a boiling water bath. The end point was then determined by titrating back with N/10 hydrochloric acid and phenolphthalein as an indicator in all cases. Five-tenths gram of Fraction C required 20 cc. of N/10 alkali. Since half a gram of a hexonic acid lactone requires theoretically 28.07 cc. of N/10 alkali, some neutral material was present, as was shown by subsequent acylation of the fraction. On the basis of this titration the remaining aqueous solution of Fraction C was, however, first digested with the calculated amount, 40.7 grams, of brucine, a small amount of alcohol being added and the mixture boiled for 1 hour on a water bath after the alkaloid had

dissolved. Removal of the water under 20–30 mm. pressure, and finally heating to 80° gave a residue of gummy brucine salts weighing 67.3 grams. After dissolving this in half its weight of water, the subsequent addition of 5 parts of alcohol gave a rapid deposition of crystal needles of *d*-mannonic brucine, 15 grams, melting from 205° to 212°. These, on recrystallization, melted at 210°–212°. Since all attempts to obtain other crystalline brucine salts from the alcoholic filtrate still containing about 50 grams of salts failed, the mixture was converted back into free brucine and acids as follows: A dilute aqueous solution of the salts was treated with an excess, about 1.5 times the calculated amount, of aqueous sodium hydroxide. The precipitated brucine was filtered off and well washed and the remainder was removed from the aqueous filtrate by threefold extraction with chloroform. The filtered alkaline solution was then treated with slightly more than the theoretical amount of aqueous hydrochloric acid and the water removed at 20–30 mm., the solution being finally heated on a boiling water bath. The organic acids were then separated from the salt by thorough extraction with absolute alcohol. In this manner 16.5 grams of acid were finally recovered.

Fraction B.—Five-tenths of a gram of Fraction B, 25 grams, required on titration a total of 37.9 cc. of N/10 alkali; the calculated amount for 0.5 gram 1,2-dioxybutyrolactone is 42.37 cc. The remainder of the aqueous solution of Fraction B was consequently converted into salts by boiling with 76 grams of brucine. Fractional crystallization of the crude salts from small amounts of water and alcohol gave first 11.7 grams of *d*-mannonic brucine melting, after recrystallization, at 210°–212°. This salt can readily be separated from the other high-melting brucine salts of *d,l*-glycerinic, erythronic and threonic acids which may also possibly be present, especially in Fractions A and B, since it comes out very rapidly from hot concentrated aqueous solutions on addition of 4 to 5 volumes of hot alcohol. The 11.7 grams salt mentioned above, as well as the 15 grams of a corresponding salt referred to under Fraction C, were now proved to consist entirely of

d-mannonic lactone as follows: Twenty-four grams of the combined salts gave with sodium hydroxide, etc., 8 grams of crude acid gum (alcohol extract) from which, on crystallization from acetic ether, a total of 5 grams of the lactone melting from 145°–148° was obtained. A portion of this, on further purification, gave crystals melting from 148°–150° and showing a specific rotation of +51.°1; *i. e.*, 2.0012 grams of substance and 18.0087 grams of water, $p = 10.001$; $d = 1.037$, found (α) in 1 dcm. tube, +5.°30.

The portion of the 8 grams of crude lactone which still remained gummy, *i. e.*, about 3.0 grams, gave on treatment with 6 cc. of 50 per cent. alcohol and 3 cc. phenylhydrazine, after 24 hours at ordinary temperatures, 3.7 grams of practically pure *d*-mannonic phenylhydrazide melting from 210° to 216°.

The mother liquors from the above mentioned *d*-mannonic brucine gave, on long standing, a total of 51 grams of crystalline salts, in three crops, which melted from 170° to 205°; and finally the 24.2 grams of gummy noncrystallizable salts left in the mother liquor gave with sodium hydroxide, etc., a total of 9.3 grams acid gum soluble in ethyl acetate. This was united with the corresponding lot, 16.5 grams, of acid gum recovered from Fraction C (see above), and the mixture then further resolved as in the first instance, by fractional precipitation with alcohol and ether, etc., into three fractions, C′, 7.1 grams of alcohol extract, least soluble acid gum; B′, 10.8 grams gum soluble in acetic ether; and A′, 5.0 grams of ether-soluble gum.

Fraction C′.—This portion, 7.1 grams, contained some tar which was removed by acylation as follows: The gum was heated for nine hours at 95°–100° with three parts of acetic anhydride; acetic acid and the excess of acetic anhydride were then removed by distillation at 20 mm. pressure, the flask was finally heated to 100°, and the residue of acylated gums taken up in hot ether, which left some insoluble tar. The acylated gums which had dissolved in the ether were then converted back into the unchanged free acids by threefold hydrolysis with three parts of water, heating for 9 hours at

100° and taking care to remove water and acetic acid after each operation by distilling at 20 mm. pressure and 100°, etc. Thus 4.4 grams of yellowish gum were recovered. Five-tenths of a gram of this required 29.6 cc. of N/10 sodium hydroxide on titration (the calculated amount for 0.5 gram hexonic acid lactone is 28.07 cc). Consequently, the remainder of the gum was converted into salts in the usual manner by digestion with 10 grams of brucine. Only one crop, 3.2 grams, of crystalline brucine salts melting at 125°–135° could be obtained from these by dissolving in alcohol, even on long standing, leaving a residue of 10.9 grams of gummy salts remaining after distillation under reduced pressure.

Fraction B′.—Five-tenths of a gram of Fraction B′ (10.8 grams) required 30 cc. of N/10 sodium hydroxide for neutralization and therefore consisted mainly of hexonic acids. On treatment of the main portion with brucine, 38.3 grams of gummy salts were obtained which, on crystallization, yielded a total of 12.2 grams of crystalline salts melting from 125° to 145°. The remainder, 21.8 grams of gummy salts, was now united with the 10.99 grams of similar salts from C′ (see above) and the whole converted by means of barium hydroxide, etc., back into 10 grams of gummy acids which obviously consisted of a mixture of hexonic acids, but which I did not succeed in separating into its components. Treatment with phenylhydrazine gave no crystallizable acid hydrazides.

Fraction A.—The first mentioned ether extract, A, 11.1 grams, was digested for 9 hours in dilute aqueous solution with 50 grams of finely ground strychnine, only 19.5 grams of the alkaloid dissolving. The gummy strychnine salts obtained after distilling off the water at 30 mm. pressure gave, after three crystallizations from absolute alcohol, a total of 17 grams of crude strychnine glycollate melting between 155° and 160°. The noncrystalline and easily soluble strychnine salts left, 18.5 grams, gave back with sodium hydroxide, etc., a total of 6.2 grams of acid gum (alcohol extract). Five-tenths of a gram of this required 39.0 cc. N/10 sodium hydroxide for neutralization (calculated for 1,2-di-

oxybutyrolactone, 42.37 cc.). The remainder gave, on digestion with 19 grams of brucine and subsequent fractional crystallization of the salts obtained, a total of 13.7 grams of brucine salts melting between 180° and 205°. Thirteen grams of easily soluble gummy salts remained which, when treated with barium hydroxide, etc., gave back 1.6 grams of acid gum. This, when converted into the strychnine salt, gave 1.2 grams more of crude strychnine glycollate melting between 150°–160°, besides 5.8 grams of gummy, easily soluble salts which were united with a corresponding lot from Fraction A′ (see below).

Fraction A′.—The second ether-soluble fraction of acid gums, 5.0 grams, took up, on boiling for 9 hours with an excess of strychnine, only 11.0 grams of the alkaloid, and fractional crystallization of the salts obtained gave in all only 2.5 grams of crude glycollic strychnine, melting between 150° and 160°. The remainder, 13.5 grams, was united with a corresponding lot of gummy strychnine salts, 5.8 grams, mentioned above under A. These gave with barium hydroxide, etc., 11.6 grams of strychnine and 5.6 grams of acid gum.

Glycollic Acid from Crude Strychnine Glycollate.—The total amount of crude glycollic strychnine obtained from Fractions A and A′ was 20.6 grams; 17.0 grams of this salt gave, with sodium hydroxide, etc., 3.0 grams of syrupy acid (acetic ether extract), and from this by digestion in aqueous solution with an excess of powdered calcium carbonate, etc., 1.9 grams of crystallized calcium glycollate, $Ca(C_2H_3O_3)_2.3H_2O$, were obtained. 0.4684 gram of the air-dried salt lost 0.1066 gram of water on drying to constant weight at 120°.

	Calculated for $Ca(C_2H_3O_3)_2.3H_2O$.	Found.
H_2O	22.13	22.75

0.7500 gram of the anhydrous calcium salt gave 0.2198 gram of CaO on ignition.

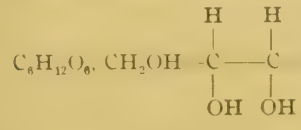
	Calculated for $Ca(C_2H_3O_3)_2$.	Found.
CaO	29.47	29.32

On the Nature of the Other Crystalline Brucine Salts Obtained.—The total amount of the various crystalline brucine salts (except *d*-mannonic brucine) obtained from the different fractions mentioned above, which had melting points varying from 125° to 205°, was 80.0 grams. These were combined and dissolved in 40 cc. hot water, and, on addition of 235 cc. of alcohol, crystals of a brucine salt melting between 180° and 190°, 21.5 grams in two crops, were obtained. Since this salt gave, on treatment with aqueous barium hydroxide, 15.4 grams of brucine and 4.6 grams of acid gum, the presence of glycerinic acid and of dioxybutyrolactone is probable but not yet established by what I have done.

A New Hexonic Acid, α-Oxymethyl-d-ribonic Acid(?).—The alcoholic mother liquors containing still about 56.5 grams of salts were found finally to give, on crystallization from 10 parts of 95 per cent. alcohol, 32.5 grams of transparent flat plates melting from 183° to 186°. These crystals are very sparingly soluble in cold water but dissolve in 10 parts of boiling water and on cooling at least 80 per cent. of the salt separates out in transparent plates. These properties are therefore so striking that this salt can very easily be separated from a mixture. The free acid obtained therefrom by aqueous sodium hydroxide, etc., and subsequent extraction of the salt residue with acetic ether, is a thick gum which gives, with an equal weight of phenylhydrazine in 2 parts of 50 per cent. alcohol, a crystalline phenylhydrazide which, on recrystallization from 50 per cent. aqueous alcohol, melts from 179° to 183°. This hydrazide was fairly difficultly soluble in cold water and practically insoluble in cold alcohol. The specific rotation of the hydrazide in 2 per cent. aqueous solution was found to be +8°.38. 0.4012 gram of the substance and 19.4591 grams of water, $p = 2.020$; $d = 1.004$; found α in a 1 dcm. tube = +0.°17. The acid gives apparently an insoluble basic barium salt since it is impossible to obtain it from its brucine salt by the usual method; *i. e.*, treatment with aqueous barium hydroxide. The resulting precipitate contains not only all the brucine, but practically all the acid in the form of an insoluble salt. I lost a considerable quan-

tity of material in this manner. Eleven grams of the brucine salt, when treated with sodium hydroxide, etc., gave 7 grams of brucine and on subsequent extraction of the salt residue with acetic ether 3.1 grams of thick acid gum were obtained. Five-tenths gram of this gum required 28.63 cc. N/10 sodium hydroxide for neutralization, where the calculated amount of N/10 alkali necessary for neutralizing 0.5 gram of a free hexonic lactone is 28.07 cc. These data prove that a hexonic acid must be present which is, however, not identical with any known hexonic acid. The calcium salt, made in the usual way from the acid gum, was found to crystallize from hot, concentrated aqueous solutions in small, translucent cubes which are very sparingly soluble in cold water. It was not analyzed, but gave in one per cent. aqueous solution a specific rotation of $+11°.98$; 0.1786 gram substance and 17.6814 grams water, $p = 1.000$, $d = 1.001$, found α in a 1 dcm. tube $= +0.°12$.

Since Mr. Spoehr, as well as Mr. Rosario,[1] have obtained an analogous new hexonic acid, α-oxymethyl-*d*-arabonic acid—in the oxidation of alkaline solutions of dextrose and laevulose by air as well as by hydrogen peroxide, it is in the highest degree probable that my acid is α-oxymethyl-*d*-ribonic acid, which must be formed from maltose by a preliminary transformation[2] into dextrose-*d*-glutose which then gives the dextrose glucosone,



This by an asymmetric benzylic acid rearrangement then gives dextrose *d*-α-oxymethylribonic acid.

The alcoholic mother liquors from which the new hexonic acid brucine salt had been removed as thoroughly as possible still contained 25.2 grams gummy brucine salts which could not be obtained in crystalline form. These were, there-

[1] Work soon to be published in the American Chemical Journal.

[2] Lobry de Bruyn and Ekenstein: Rec. Trav. Chim., **16**, 258, 274. Nef: Ann. Chem. (Liebig), **357**, 297.

fore, decomposed with barium hydroxide into 16 grams of brucine and 9.0 grams of acid gum, indicating therefore, the presence of other hexonic acids.

Brief Summary of Results of Other Experiments with Maltose and Fehling's Solution.—An experiment with Fehling's solution on 144 grams maltose carried out in practically the same manner as given in detail above gave 133.7 grams of thick, syrupy glucosido acids which on hydrolysis gave 66.7 grams of gummy acids and 55.04 grams of dextrose. Forty-four and seven-tenths grams of the acids dissolved in acetic ether on thorough digestion, leaving, therefore, 22.2 grams of insoluble gum. The ethyl acetate solution deposited on standing 6.5 grams of crystallized mannonic lactone. On treating the gummy acids, 38.2 grams, recovered from the solvent, with 40 cc. alcohol and 38 cc. phenylhydrazine for 24 hours at ordinary room temperature, 9.45 grams of crystalline hexonic acid hydrazides melting from 204° to 206° separated out. On similar treatment with phenylhydrazine the 22.2 grams of gummy acids insoluble in acetic ether gave 25.5 grams of crystalline acid hydrazides. These combined hydrazides, 35 grams, gave on hydrolysis with 7 per cent. sodium hydroxide,[1] 13.05 grams of mannonic lactone, besides 5.5 grams of gummy acids. A portion of the latter when titrated with N/10 alkali gave results agreeing with those calculated for a free hexonic acid, and on converting the main portion of the gum with the calculated amount of brucine into salts (16.8 grams) there were obtained on fractional crystallization from aqueous alcohol 10.0 grams of mannonic brucine and 5.8 grams of the new so-called *α*-oxymethyl-*d*-ribonic brucine melting at 181°–183°. This result indicates that *d*-gluconic acid cannot be present in any appreciable quantity. It must, however, be emphasized in this connection that treatment of the various fractions of the acid gums with phenylhydrazine and alcohol never removes the hexonic acids completely in the form of insoluble hydrazides. I obtained, for instance, in working up the acids forming alcohol-soluble hydrazides in the experiment just referred to above

[1] Nef: Ann. Chem. (Liebig), **357**, 225.

(by hydrolysis, etc.), a total of 21.0 grams of the very characteristic α-oxymethyl-*d*-ribonic brucine melting from 181° to 183°.

It is apparent, therefore, that the complete separation of the various hexonic acid hydrazides, which by themselves are practically insoluble in cold or hot absolute alcohol, is prevented to a considerable extent by the presence of the other acids which form alcohol-soluble hydrazides or phenylhydrazine salts.

Summarizing finally, there were obtained from 136.8 grams of anhydrous maltose in the reaction described in detail above 10.6 grams of carbon dioxide, 4.74 grams of formic acid, and 132.8 grams of nonvolatile gums consisting chiefly of glucosido acids. The latter gave on hydrolysis, besides 47.5 grams of dextrose, 82.35 grams of nonvolatile acids. A total of 45.02 grams of these acids was identified quantitatively as follows: 0.35 gram oxalic acid, 3.92 grams glycollic acid, 30.25 grams *d*-mannonic lactone, and 10.5 grams of a new hexonic acid called α-oxymethyl-*d*-ribonic acid. The nature of the remaining 37 grams unidentified acids was not established, mainly because of lack of time.

Experiments with d-*Maltosone and* d-*Glucosone.*—It is extremely probable, from my results with maltose, that only two hexonic acids, namely, *d*-mannonic and α-oxymethyl-*d*-ribonic acid, are obtained in the hydrolysis of the glucosido acids which are formed by the action of Fehling's solution on maltose. In that case, both acids must be formed by an asymmetric benzylic acid rearrangement from 1,2-maltosone and 2,3-maltosone, respectively.[1] 1,2-*d*-Glucosone, which must be the common intermediate product when *d*-glucose, *d*-fructose, and *d*-mannose are oxidized by Fehling's solution, gives, on the other hand, by a partial asymmetric benzylic acid rearrangement, mainly *d* gluconic acid and only small amounts of *d*-mannonic acid.[2]

It was, therefore, a point of great interest to prove the correctness of these conclusions by actually studying the

[1] Nef: Ann. Chem. (Liebig), **357**, 281 and 282.
[2] *Ibid.*, **357**, 284.

behavior of caustic alkalies towards *d*-glucosone as well as *d*-maltosone, substances which are now not so very difficult of access.[1] My preliminary experiments tend to confirm the correctness of the conclusions reached, but I regret to have to admit that the results are not absolutely final or decisive, and can only become so by a very careful repetition of the work with far larger quantities of material.

Experiments with Maltosone.—Maltosone was prepared from the osazone by the method of Emil Fischer and Armstrong.[2] One hundred and thirty grams of maltose gave, on treatment with aqueous phenylhydrazine acetate, 92.1 grams of maltosazone melting at 210°. On treating this, according to the directions, in 20-gram lots with benzaldehyde, I never obtained more than 11.0 grams of crude maltosone from 45 grams of osazone instead of the calculated 29.4 grams. That this poor yield is due chiefly to an incomplete hydrolysis of maltosazone under the conditions employed by Fischer and Armstrong was proved by subsequent experiments in which an almost quantitative yield was obtained by heating for 10 hours longer and treating a second time, after concentrating the aqueous filtrate to a small volume, with more benzaldehyde. Thus a mixture of 19 grams of maltosazone in 100 parts of boiling water gave finally, after 10 hours' heating on the boiling water bath with 1.5 parts of benzaldehyde, 11.9 grams of crude maltosone, instead of the calculated 12.4 grams. It is, of course, possible that some maltosone had been split into glucosone and dextrose on account of the prolonged heating with boiling water.

The crude maltosone prepared exactly according to Fischer and Armstrong's directions showed the following properties: (*a*) it gave on treatment in the cold with phenylhydrazine acetate an equal weight of maltosazone, crystallizing in rosettes, melting at 210°, and entirely free from the large, flat, needle-shaped crystals of *d*-glucosazone.[3] (*b*) A solution of 0.2 gram of maltosone in 4 cc. of water, when added to 5 cc. of Fehling's solution, was markedly reduced in one minute, completely

[1] Ber. d. chem. Ges., **35**, 3141.
[2] *Loc. cit.*
[3] J. Am. Chem. Soc., **25**, 1015. Grimbert: J. Pharm. Chim., **7**, 17–5.

in one-half hour in the cold. If, however, the maltosone was first dissolved in an alkaline tartrate solution, and this mixture allowed to stand 48 hours before the addition of the copper sulphate solution, the same conditions as to concentration and amounts being observed, only slight reduction to cuprous oxide was noticeable, approximately only 8 per cent. of that in the first experiment. (*c*) Cold solutions of 20 grams of maltosone in 400 cc. of water and of 18.5 grams of crystallized barium hydroxide in 200 cc. of water were mixed and allowed to stand for 48 hours. The calculated amount of sulphuric acid was then added to the dark red solution, which contained also a flocculent precipitate. In order to hydrolyze the supposed dextrose-*d*-mannonic acid formed, the filtrate was then made up to 1 liter with water, 50 grams of sulphuric acid added and the solution boiled five hours. The free sulphuric acid was now removed in the usual manner, care being taken to leave only a trace of barium in the solution. The water was removed by distillation under reduced pressure and the residue, 11.5 grams, was taken up in hot absolute alcohol. On standing, the characteristic crystals, 0.3 gram, of mannonic lactone separated out. These melted, on recrystallization from acetic ether, between 148°–152°. The rest of the acid gum obtained, 11 grams, was resolved by solution in 20 cc. of alcohol and partial precipitation by addition of 40 cc. ether, into 2 fractions, *i. e.*, 4.8 grams soluble and 6.2 grams insoluble gum. The latter gave, on heating for 8 hours with 19 cc. acetic anhydride at 100°, 10.2 grams of acylated gum of which only 6.8 grams dissolved in hot ether. The latter, on fourfold hydrolysis with five parts of water, finally gave back 4.2 grams of acid gum. Twenty-five hundredths gram of this required 21.1 cc. of N/10 sodium hydroxide for neutralization. The remainder was then converted by means of 13.3 grams brucine into 18.5 grams gummy brucine salts. These gave, on fractional crystallization from alcohol, first 2 grams of crude *d*-mannonic brucine melting from 180° to 190°, which was converted by means of sodium hydroxide into the crystalline lactone melting at 145°–148°, as well as its phenylhydrazide melting at 200°–205°, and

second, 1.3 grams melting from 138° to 140°. This, on crystallization from 1.5 cc. water, gave transparent crystalline plates melting between 179° and 180°, and resembling very closely the so-called α-oxymethyl-*d*-ribonic brucine which I obtained from maltose by means of Fehling's solution. Finally, the gummy brucine salts left gave back 2.6 grams of acid gum which, on treatment with phenylhydrazine and alcohol, gave 1.2 grams of crystalline hydrazides melting from 185° to 195°.

Experiment with Glucosone.—Twenty-three and seven-tenths grams of crude maltosone, prepared according to the modified method given above from 38 grams of maltosazone, was hydrolyzed with 5 parts of 5 per cent. sulphuric acid for 5 hours in a boiling water bath. Considerable charring took place in this operation and on freeing the solution, first from sulphuric acid and then from water, 20 grams of a mixture of glucose and glucosone gum were obtained. This gave the following tests: Reduction took place at once with Fehling's solution in the cold. The presence of free glucose was shown by the production of gas with yeast suspension in the fermentation tube, since glucosone does not ferment with yeast.[1] One gram of the gum, when treated with phenylhydrazine acetate, gave rapidly, at room temperature, 0.5 gram of crystalline glucosazone, melting at 205°. The filtrate, on boiling with 0.5 gram of phenylhydrazine and 0.5 cc. of 50 per cent. acetic acid, gave a further crop of *d*-glucosazone, the presence of both 1,2-glucosone and *d*-glucose being thus indicated.[2] The remaining 18.5 grams of gum was now treated in the cold with one molecule of barium hydroxide, 17.2 grams, and 555 grams of water, giving therefore approximately the same concentration as was employed in the corresponding maltosone experiment described above, and set aside for 24 hours. Darkening and deposition of a flocculent precipitate was noticed and after removal of all but traces of the barium present, the water was distilled off under reduced pressure, a residue of 17.0 grams acid gum being

[1] Fischer and Armstrong: Ber. d. chem. Ges., **35**, 3144.
[2] E. Fischer: Ber. d. chem. Ges., **23**, 2121.

left. Three and seven-tenths grams of this which dissolved in ether were rejected and the remaining 13.3 grams gum were acylated in the usual manner, 21.8 grams of acylated gums being obtained, of which only 10.6 grams were soluble in hot ether, and fourfold hydrolysis of these with water at 100° gave finally 9.0 grams of acid gum. Five-tenths of a gram of the gum required 32.6 cc. of N/10 sodium hydroxide for neutralization (calculated for pentonic acid lactone, 33.8 cc). The remainder was then converted into brucine salts, which on crystallization by long standing gave finally 12.4 grams of crystalline salts melting from 135° to 145°. These gave back with sodium hydroxide, etc., 2.0 grams of acid gum, of which 0.5 gram, when treated with phenylhydrazine in aqueous alcohol solution, gave 9.5 grams of an acid hydrazide melting from 190° to 195°. The specific rotatory power of this in one per cent. solution was found to be $+12°.27$; *i. e.*, 0.20 gram substance in 19.8 grams water, $p = 1.0$, $d = 1.002$, found α in 1 dcm. tube $= +0°.12$. This indicates the presence of *d*-gluconic acid since a known sample of *d*-gluconic hydrazide melting at 198° was found to give, in 2 per cent. aqueous solution, $[\alpha]_D^{20} = +13°.0$.

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